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Field testing of antagonists of Fusarium head blight incited by *Gibberella zeae*

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Abstract

Fusarium head blight (FHB), primarily caused by *Gibberella zeae* (anamorph = *Fusarium graminearum*), is a devastating disease that causes extensive yield and quality losses to wheat and barley throughout the world. Antagonists that suppressed FHB in earlier greenhouse studies were tested in a series of field experiments at different geographical locations in the United States in 1998–2000. In most cases, the yeasts *Cryptococcus nodaensis* OH182.9, *Cryptococcus* sp. OH 71.4, and *Cryptococcus* sp. OH 181.1 decreased disease severity throughout the study at all locations. The most effective antagonists reduced disease severity by as much as 50–60%. The efficacy of some antagonists differed depending on the dose applied, but differences were not necessarily related to a dose–response. Antagonist biomass produced in two liquid culture media with differing carbon to nitrogen (C:N) ratios suppressed FHB disease severity. On the susceptible winter wheat cultivar Pioneer 2545 at Peoria, IL, in 2000, yeast OH 182.9 reduced disease severity by 60% and by 45%, compared to the buffer control when produced in C:N 11.0 and 6.5 medium, respectively. The influence of C:N ratio of the production medium on antagonist efficacy on cultivar Pioneer 2545 varied with the antagonist considered but did not influence disease reduction on the moderately resistant winter wheat cultivar Freedom at the Peoria, IL or Wooster, OH, locations. The lowest levels of disease of any study occurred when antagonists were applied to this moderately resistant cultivar. Biological control of FHB offers a potentially useful tool for inclusion in an IPM program for combating FHB on winter and spring wheats. Published by Elsevier Inc.

Keywords: *Fusarium graminearum*; Scab of wheat; Biocontrol; Bacteria; Yeasts

1. Introduction

Fusarium head blight (FHB) is responsible for extensive damage of wheat in humid and semi-humid regions of the world (Bai and Shaner, 1994; McMullen et al., 1997). The primary causal agent of this disease, *Gibberella zeae* (Schwein.) (anamorph = *Fusarium graminearum* (Schwabe)) can produce potent toxins such as the estrogenic toxin zearalenone (F-2) (Vesonder and Hesseltine, 1980) and the trichothecene deoxynivalenol (DON, vomitoxin) (Proctor et al., 1995; Snijders, 1990) during the colonization of grain, and in some cases, during storage (Homdork et al., 2000). DON is water soluble and can inhibit amino acid incorporation and protein production in plant tissues (Casale and Hart, 1988). Grain heavily contaminated by the toxin is frequently unsuitable for human consumption and can cause emesis and feed refusal in animals (Forsyth et al., 1977; Vesonder et al., 1976). DON has been regulated by United States Food and Drug Administration (FDA) at 1 μg/g for finished flour products (Windels, 2000), but foreign buyers can impose their own standards that can be more stringent than the FDA (Hawk, 1988). Registered fungicides can be effective against FHB (Jones, 1999, 2000; Suty and Mauler-Machnik, 1997; Wilcoxson, 1996), however, residues, costs, and the possibility of some chemicals indirectly increasing the DON content of grain are concerns with chemical usage (Pirgozliev et al., 2002). Commercial wheat cultivars that show a high degree of resistance are not currently available (Bai et al., 2000; Bushnell et al., 1998; Johnston, 1994). Although conventional tillage

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may be a means for reducing FHB, the preference for minimal tillage agriculture renders this alternative less attractive (Bai and Shaner, 1994; Dill-Macky and Jones, 2000; Miller et al., 1998; Teich and Hamilton, 1985).

Biological control of FHB has shown promise in previous studies. Application of *Trichoderma harzianum* (Fernandez, 1992) or *Microsphaeropsis* sp. (Bujold et al., 2001) on wheat straw in the field has been used to advance decomposition of the debris and preclude colonization and inoculum production by *G. zeae*. Studies of in vitro antagonism of *G. zeae* growth were initiated in Brazil (da Luz, 1988). Perondi et al. (1996) reported successful biocontrol by applying antagonists to flowering wheat fields in Brazil, and similar studies have been reported in China (Fuming et al., 1994). We have reported effective biological control of FHB on durum wheat in greenhouse and field studies (Schisler et al., 2002) and on hexaploid wheat using several different antagonists in greenhouse, but not field, assays (Khan et al., 2001). The effectiveness of biological control agents can vary with the host cultivar employed (King and Parke, 1993). The wheat cultivar Freedom is a moderately resistant cultivar while Pioneer 2545 is susceptible to developing FHB. Whether FHB can be reduced to a greater extent by combining host resistance and the use of biocontrol agents has not been tested. The effect of antagonist dosage on performance has been studied in other pathosystems (Johnson, 1994; Schisler et al., 1997; Smith et al., 1997). The influence of antagonist dosage on biocontrol efficacy has not been studied in FHB biocontrol systems. The efficacy of antagonists can be affected by production media (Schisler et al., 2000b; Slininger et al., 1994). Using a low carbon to nitrogen (C:N) ratio medium to produce some yeasts has been shown to improve their survival (Beker and Rapoport, 1987). Whether the C:N ratio of the medium used to produce antagonists of FHB would influence antagonist efficacy has not been investigated. Ultimately, optimizing production media, antagonist dose, and utilizing a resistant host cultivar should enhance the level of FHB control that can be obtained in field environments. It is understood, however, that biological control can be variable in field environments which can complicate the interpretation of treatment effects. The influence of antagonist dosage and biomass production media may therefore be difficult to discern.

Our objectives for this study were to (1) test the efficacy of antagonists of *G. zeae* on hexaploid wheat in the field, (2) ascertain whether symptoms of FHB can be minimized by utilizing FHB antagonists on a wheat cultivar resistant to FHB, (3) determine the effect of antagonist dosage on suppression of FHB, and (4) determine the effect of the C:N ratio of the antagonist production medium on antagonist efficacy in suppressing FHB. Preliminary results from some of these studies have been reported (Boehm et al., 1999; Schisler et al., 2000a).

2. Materials and methods

2.1. Antagonist inoculum production

Bacillus subtilis AS 43.3, *B. subtilis* AS 43.4, *Cryptococcus* sp. OH 71.4, yeast OH 72.4, *B. subtilis* OH 131.1, *Cryptococcus* sp. nov. OH 181.1, and *Cryptococcus nodaensis* OH 182.9 were used in at least one study (Table 1). Strains stored at −80 °C in 10% glycerol were streaked for purity on 1/5 Tryptic soy broth agar (TSBA/5, pH 6.8) (Difco Laboratories, Detroit, MI) and incubated at 25 °C for 24 h. Cells of individual strains were harvested and used to seed 100 ml of semi-defined complete liquid medium (SDCL, Slininger et al., 1994) contained in 500-ml flasks (OD of 0.10 at A_{620} (Khan et al., 2001)). Flasks were incubated at 25 °C at 250 rpm (2.5 cm eccentricity) for 24 h. Unless otherwise stated, the C:N ratio of the SDCL medium was 11.0. After 24 h, pre-cultures were used to seed Fernbach flasks containing 1.5 liters of SDCL to an OD of 0.10. Cultures were incubated for 48 h at 25 °C and 250 rpm. Colonized broth was then transferred to sterile containers, transported to the field on ice, and used within 24 h.

2.2. Pathogen inoculum production

In 1998 field experiments at Peoria IL, *G. zeae* (Z 3639) inoculum was grown on V8 juice agar as described

Table 1
Antagonist strain designation and identification of bacteria and yeasts that reduce the severity of Fusarium head blight of wheat

Antagonist	NRRL Accession No.[a]	Identification
AS 43.3	B-30210	*B. subtilis/amyloliquefaciens*[b]
AS 43.4	B-30211	*B. subtilis/amyloliquefaciens*[b]
OH 71.4	Y-30213	*Cryptococcus* sp. (= *Torula aurea*)[c]
OH 72.4	Y-30214	Yeast[d]
OH 131.1	B-30212	*B. subtilis*[e]
OH 181.1	Y-30215	*Cryptococcus* sp. nov.[c]
OH 182.9	Y-30216	*C. nodaensis*[f]

[a] ARS (NRRL) patent culture collection, National Center for Agricultural Utilization Research, Peoria, IL.

[b] Identification by Deutsche Sammlung von Mikroorganismen und Zelkulturen GmbH (DSMZ), Braunschweig, Germany, based on 16S rDNA sequence homologies and biochemical and physiological tests of taxonomic utility.

[c] Identification based on nucleotide sequence divergence in domain D1/D2 of large subunit 26S rDNA and on divergence in internal transcribed spacer (ITS) 1/5.8/ITS2 rDNA. C.P. Kurtzman, *personal communication*.

[d] Identification not determined.

[e] Identification by MIDI Labs, Newark, DE, based on 16S rDNA sequence homologies and biochemical and physiological tests of taxonomic utility.

[f] Identification based on nucleotide sequence divergence in domain D1/D2 of large subunit 26S rDNA and on divergence in ITS 1/5.8/ITS2 rNNA (C.P. Kurtzman, *personal communication*, and Sato et al., 1999).

previously (Khan et al., 2001). Harvested conidia ($\sim 5 \times 10^4$ CFU/ml) were mixed with cells of an antagonist and the suspension sprayed on flowering heads of wheat. In 1999–2001 experiments at Peoria, IL, *G. zeae* was grown on yellow dent corn kernels contained in 3- or 4-liter Erlenmeyer flasks. Kernels were soaked in distilled water for 24 h, autoclaved for 1 h on two consecutive days, and inoculated with 10 ml of a conidial suspension of *G. zeae* containing approximately 2×10^5 CFU/ml. Upon completion of autoclaving, the swelled corn kernels filled one-half of the volume of a flask. Flasks were kept at room temperature on a laboratory bench for 2 weeks and shaken periodically. The colonized corn kernels were then spread in the field at approximately 30 g/m^2. Kernel application took place approximately 2.5 weeks before the flowering date of the wheat. Perithecia appeared on the kernels 1 week after being applied to fields. At Wooster, OH, approximately 0.8 kg of yellow dent corn kernels were placed into 3.8-liter plastic milk bottles with 0.6 liters of tap water. Bottles were closed with cotton plugs, covered with aluminum foil, and allowed to stand for 24 h. They were then autoclaved for 1 h on two consecutive days. Bottles were shaken to loosen kernels before and after each autoclaving. Strips of PDA agar colonized by *G. zeae* Fg 3-93 and Fg 6-93 were placed into the bottles and shaken to ensure even distribution. Bottles were maintained on a laboratory bench for 2 weeks and shaken every other day to permit thorough colonization. Three weeks before anthesis, colonized kernels were broadcast within wheat plots at a rate of approximately 30 g/m^2. In the 2000 trial at the Langdon, ND field site, naturally occurring levels of *G. zeae* incited disease.

2.3. Efficacy of putative antagonists: 1998 field trial at Peoria, IL

Three *Bacillus* sp. and four yeast isolates (Table 1) were screened on soft red winter wheat cultivar Pioneer 2545 (susceptible to FHB). Antagonist biomass was produced in liquid culture in Fernbach flasks containing SDCL medium as described above. The field site soil was an Orthents complex, with a silty loam surface layer of approximately 25 cm and an underlying silty clay loam. The site was conventionally cultivated in the fall prior to fall planting of the wheat. Prior to planting, the site was fertilized with 1120 kg/ha of Parker's Super Soilife 10–10–10 (Pursell Industries, Sylacauga, AL) (3.92% ammonium nitrate and 6.68% urea nitrogen (1.7% slow release)) and again conventionally cultivated. Rows of Pioneer 2545 (2.1 m long) were planted by hand with 0.3 m between rows. Rows were assigned to treatments using a randomized, complete block design with four replicate rows per treatment. A border row of Pioneer 2545 surrounded the experiment site and was not treated. Prior to application to flowering wheat heads, 48 h, colonized culture broths were diluted to 25% of full strength using weak, pH 7.2 phosphate buffer (PO_4 buffer) (0.004% w/v KH_2PO_4 buffer with 0.019% w/v $MgCl_2$). Tween 80 was added to microbial suspensions to a final concentration of 0.036% (v/v). Final colony forming units per milliliter counts for antagonist treatments were approximately 5×10^8 and 3×10^7 CFU/ml for bacteria and yeast strains, respectively.

Treatment suspensions were applied using a CO_2 backpack sprayer charged at 2.8 kg/cm^2 and equipped with two, #10 cone-jet nozzles (R&D Sprayers, Opelousas, LA) spaced 30 cm apart and mounted pointing inward at 45°. Treatment suspensions were charged with CO_2 just prior to application. Treatments were applied to near run-off just prior to and continuing after sunset to minimize potential UV degradation of antagonist cells. The primary control treatment consisted of plants treated with a solution of buffer/Tween 80. A second control consisted of untreated plants. Treatment applications were confined to individual rows using two PVC pipe frames covered by plastic that were placed on either side of a row to be treated. The PVC pipe frames were thoroughly rinsed after each treatment application. From the morning after treatment application until mid-milk kernel development (Feekes growth stage 11.1, (Large, 1954)), wheat heads were misted with city water for 5 min/h from 6:00 AM to 8:00 PM using four misting sprinklers (Sherman PTR-111 sprinkler, L.R. Nelsen, Peoria, IL) spaced evenly across the plot with misting heads set approximately 10 cm above wheat heads, resulting in a total uniform application of approximately 1.5 cm of water/day.

When plants were at late milk development, field assessments of FHB incidence and severity (Stack and McMullen, 1995) were made by evaluating 60 heads per replicate (240 heads/treatment). When grain reached full maturity, wheat heads were harvested by hand and threshed using an Almaco small vogel plant and head thresher (Nevada, Iowa) set at the lowest air setting to retain all kernels regardless of their disease status. Grain samples obtained from each replicate row were evaluated for 100 kernel weight and a 10–20 g sample evaluated for DON content using a Veratox 5/5 quantitative DON Test Kit (Neogen, Lansing, MI) per the manufacturer's instructions for grain with a DON concern level of 0.5–5 ppm. Disease data were normalized when needed using the arcsine transformation before analysis of variance (ANOVA). Means were separated from the controls at $P \leqslant 0.05$ using Fisher's protected LSD test (FPLSD; PC SAS, version 6.12, SAS Institute, Cary, NC).

2.4. Efficacy of putative antagonists: 2000 field trial at Langdon, ND

The field site was a Svea loam that was chisel plowed the fall prior to spring planting of hard red spring wheat

cultivars Grandin and Russ. Fifty-six kilogram per hectare urea (46 + 0 + 0) and 78.4 kg/ha 11 + 52 + 0 were broadcast on the plot prior to planting and the site was cultivated conventionally. Seeding was done by an Almaco seeder to plant plots in a randomized complete block design (RCBD) with six replications per treatment. Plots were 1.05 by 1.05 m, had a 0.15 m row spacing, and were separated by 1.05 m. Border plots were not included in the experiment. Additional nitrogen was broadcast applied at the two-leaf stage in the form of 112.1 kg/ha urea. The experiment was conducted as described for Peoria, with the following exceptions. Infection of heads by *G. zeae* was dependent on naturally occurring levels of inoculum and rainfall. Biomass of antagonists AS 43.3, AS 43.4, OH 71.4, OH 131.1, OH 181.1, and OH 182.9 was produced in SDCL (C:N 6.5) and stored for 24 h on ice prior to application. SDCL (C:N 6.5) was obtained by decreasing glucose and increasing Casamino acid content of the medium while maintaining constant carbon loading. Field assessments of FHB incidence and severity were made by evaluating 60 heads per replicate (360 heads/treatment). At full maturity, wheat heads were harvested using a Hege plot combine (Hege Maschinen, Waldenburg, Germany) set on the lowest blower setting to avoid removing lighter weight kernels infected by *G. zeae*.

2.5. Effect of antagonist dosage: 1999 Peoria, IL, and Wooster, OH, field tests

Three *Bacillus* sp. and four yeast isolates (Figs. 1 and 2; Table 3) were screened on soft red winter wheat cultivars Pioneer 2545 (susceptible to FHB) and Freedom (moderately resistant to FHB). Cultivar Freedom was selected for use in an attempt to acquire data regarding the integration of biocontrol and genetic resistance for managing FHB. Antagonists were produced in liquid culture in Fernbach flasks containing SDCL medium (C:N 11.0) as described above and applied at the beginning of flowering in aqueous suspensions as explained above. Bacterial suspensions contained either 10 or 50% fully colonized liquid culture or approximately 2×10^8 and 1×10^9 CFU/ml, respectively. Yeast suspensions contained either 10 or 50% liquid culture or approximately 1×10^7 and 5×10^7 CFU/ml, respectively. At Peoria, field preparations and operations were the same as explained above.

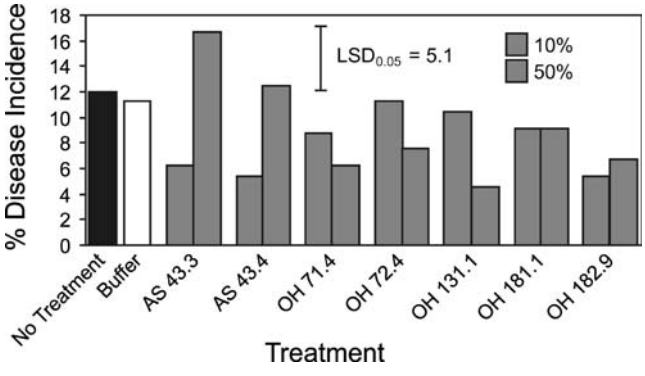

Fig. 1. Efficacy of two dosage rates of antagonists in suppressing FHB disease incidence in cultivar Pioneer 2545 in a 1999 Peoria, IL, field experiment. Wheat heads were sprayed to near run-off at the time of flowering with a suspension of 48 h old antagonist-colonized broth (broth C:N = 11.0) diluted to 50 or 10% with weak PO_4 buffer and Tween 80 wetting agent. The "Buffer" treatment was sprayed to run-off with weak buffer and Tween 80. All treatments were subjected to infection by ascospore inoculum from natural sources and *G. zeae* colonized yellow dent corn.

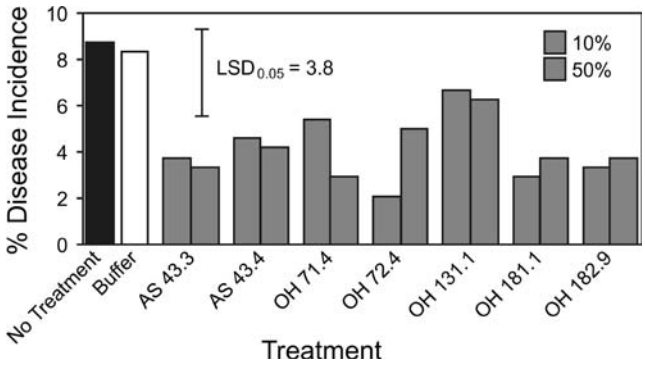

Fig. 2. Efficacy of two dosage rates of antagonists in suppressing FHB disease incidence in cultivar Freedom in a 1999 Peoria, IL, field experiment.

Prior to planting at Wooster, OH, the experimental site was moldboard plowed, granular fertilizer (19.1 kg N/ha, 80.6 kg P/ha, and 80.6 kg K/ha) broadcast over the field and the fertilizer incorporated with a disc. An additional 67.2 kg N/ha was applied in the spring as ammonium nitrate. A 3-m wide border of winter rye was planted to surround the entire nursery. Six-row experimental units, 1.3 m long and 0.19 m between rows, were planted with a Hege 1000 Series plot planter. The nursery was mist irrigated approximately 2 weeks prior to anthesis and continued until 3–4 days after anthers had been shed. Mist was applied to the canopy daily for 2.5 min of every 10 min from 6:00 to 10:00 AM and 8:00 to 10:00 PM. The mist irrigation system consisted of 0.9 m tall risers with two mist nozzles at the end of 1.54 m wide arms. Risers were spaced every 2.4 m. Nozzles were NAAN 7110 Series Bridge with a mist sprayer head (no. 327122) and a nozzle (no. 5920910) with a 0.89-mm opening (Waldo and Associates, Perrysburg, OH). The nozzle output was 38.6 liters/h at 2.8 kg/cm^2 covering a 2-m diameter area. Triademafon (122 g a.i. in 946 liters/ha) was applied as a foliar spray at early boot growth stage to control powdery mildew (*Blumeria graminis* f. sp. *tritici* (DC. Em. Marchal)) and Stagonospora glume blotch (*Stagonospora nodorum* (Berk.) E. Castell. & Germano).

Plots were scored for disease severity and incidence, harvested to determine 100 kernel weights and samples were analyzed for DON concentration as described earlier. There were four replications (2.1 m rows) at Peoria, IL and six (1 m^2 plots) at Wooster, OH. After ANOVA, data from the control treatments were removed and disease severity and incidence data analyzed as a 2×7 complete factorial to determine if dosage or antagonist treatments differed in their influence on FHB.

2.6. C:N ratio of the production media for antagonists: 2000 field trials at Peoria, IL, and Wooster, OH

Inoculum of seven microbial antagonists (Tables 1, 4, and 5) effective against FHB was produced using two SDCL media (C:N 11.0 and C:N 6.5), both with a carbon content of 14.0 g/liter. These media were selected in part because of data obtained from a preliminary field trial on durum wheat in which antagonists produced in the C:N 6.5 medium were 18% more effective in reducing FHB than the same antagonists produced in the C:N 11.0 medium (Schisler, unpublished). The field preparations and other operations were the same as explained above. The soft red winter wheat cultivars Pioneer 2545 and Freedom were used in both locations. Biomass was harvested from Fernbach shake flasks as described previously and applied at the beginning of wheat flowering in aqueous suspensions contained 20% fully colonized broth (approximately 5×10^8 CFU/ml for bacteria and approximately 2.5×10^7 CFU/ml, for yeasts). Controls were untreated plants and plants treated with PO_4 buffer/Tween 80 only. Plots were scored for disease severity and incidence, 100 kernel weight, and DON content of grain. Randomized complete block designs were used in both trials with four replications (2.1 m rows) at Peoria, IL, and six (1 m^2 plots) at Wooster, OH. After ANOVA, controls were removed and the disease severity and incidence data analyzed as a 2×7 factorial to determine if C:N or antagonist treatments differed in their influence on FHB.

3. Results

3.1. Efficacy of putative antagonists: 1998 Peoria, IL, field test

Due to cold weather at the time of flowering, disease severity was minimal (<6%). Although no significant reduction in disease severity was observed with any of the antagonists tested, wheat treated with every antagonist except yeast OH 181.1 had significantly reduced FHB incidence ($P \leqslant 0.05$, FPLSD) compared with the 'no treatment' control at 35% incidence. FHB incidence was reduced by 50% for wheat treated with yeast OH 71.4 while reductions in incidence of 36% occurred in wheat treated with bacteria AS 43.4 and yeast OH 182.9.

3.2. Efficacy of putative antagonists: 2000 field trial at Langdon ND

In plots of cultivar Grandin, wheat treated with five of six antagonists had lower FHB severity and incidence compared to the "no treatment" control with OH 181.1 and OH 182.9 treated wheat displaying reductions in severity of 53 and 44%, respectively (Table 2). Treatments with bacterial antagonist AS 43.3 and yeasts OH 71.4 and OH 181.1 had increased 100 kernel weight compared with the "no treatment" control (Table 2). In variety Russ (Table 2) only wheat treated with AS 43.4 and OH 182.9 had reduced disease severity compared to the "no treatment" control and no antagonist treated wheat had increased 100 kernel weight. Inexplicably, in both cultivars the buffer and

Table 2
Efficacy of antagonists in reducing FHB on cultivars Grandin and Russ at Langdon, ND, in a 2000 field experiment[a,b]

Treatment	Grandin			Russ		
	DS (%)	DI (%)	100-kw (g)	DS (%)	DI (%)	100-kw (g)
No treatment	21.8	52.8	3.36	7.0	21.7	3.43
Buffer	12.9	28.1	3.57	4.7	17.0	3.42
AS 43.3	16.6	39.7	3.44	7.1	24.4	3.49
AS 43.4	14.3	42.2	3.48	4.0	13.6	3.41
OH 71.4	18.9	47.8	3.55	5.5	18.9	3.48
OH 131.1	12.7	42.8	3.34	6.8	23.1	3.47
OH 181.1	10.4	32.5	3.49	5.6	16.1	3.43
OH 182.9	12.3	37.2	3.42	4.6	15.3	3.34
$LSD_{(0.05)}$	3.6	6.4	0.11	2.4	5.5	0.07

[a] Wheat heads were sprayed to near run-off at the time of flowering with a suspension of 48-h-old antagonist-colonized broth (broth C:N = 6.5) diluted to 25% with weak PO_4 buffer and Tween 80 wetting agent. Microbial suspensions contained approximately 3×10^7 and 5×10^8 CFU/ml for yeast (OH 71.4, OH 181.1, and OH 182.9) and bacterial (AS43.3, AS 43.4, and OH 131.1) antagonists, respectively. The "Buffer" treatment was sprayed to run-off with weak buffer and Tween 80. All treatments were subjected to infection by ascospore inoculum from natural sources.

[b] DS, disease severity; DI, disease incidence; and 100-kw (g), 100 kernel weight. Within a column means are compared using the $LSD_{(0.05)}$ value at the bottom of the column (Fisher's protected LSD, $P \leqslant 0.05$).

Table 3
Efficacy of antagonists in reducing FHB on cultivars Pioneer 2545 and Freedom at Wooster, OH, in a 1999 field experiment[a,b]

Treatment	Pioneer 2545				Freedom			
	DS (%)	DI (%)	100-kw (g)	DON (ppm)	DS (%)	DI (%)	100-kw (g)	DON (ppm)
No treatment	10.3	33.6	2.89	6.4	2.9	18.2	2.99	0.8
Buffer	11.0	34.4	2.76	9.9	3.5	17.8	2.89	0.9
AS 43.3 (10%)	9.8	31.4	2.93	10.3	2.2	18.1	2.93	1.3
AS 43.3 (50%)	10.3	33.9	2.78	11.7	3.2	20.6	2.84	1.0
AS 43.4 (10%)	8.5	33.6	2.92	8.6	2.6	16.4	2.91	0.8
AS 43.4 (50%)	10.7	38.1	2.76	11.2	4.0	28.9	2.80	1.0
OH 71.4 (10%)	4.8	23.1	2.94	6.0	1.6	12.5	2.88	0.8
OH 71.4 (50%)	9.6	30.6	2.85	7.2	3.0	17.2	2.81	0.7
OH 72.4 (10%)	10.7	34.4	2.95	11.2	3.2	22.0	2.85	0.9
OH 72.4 (50%)	5.3	20.3	2.98	5.8	1.7	14.5	2.86	0.6
OH 131.1 (10%)	6.5	25.3	2.87	6.4	3.0	21.4	2.91	1.0
OH 131.1 (50%)	5.1	22.0	2.93	7.8	2.0	16.7	2.86	0.9
OH 181.1 (10%)	6.7	27.8	2.83	9.0	2.3	15.3	2.94	0.7
OH 181.1 (50%)	6.3	25.3	2.87	7.0	4.5	24.4	2.97	1.0
OH 182.9 (10%)	4.6	23.1	2.98	5.7	3.7	16.1	2.84	0.7
OH 182.9 (50%)	7.1	27.0	2.96	8.0	2.8	20.8	2.94	0.8
$LSD_{(0.05)}$	2.6	6.6	0.10	4.1	1.4	5.7	0.10	0.7

[a] Wheat heads were sprayed to near run-off at the time of flowering with a suspension of 48-h-old antagonist-colonized broth (broth C:N = 11.0) diluted to 50 or 10% with weak PO_4 buffer and Tween 80 wetting agent. Microbial suspensions that were diluted to 50% contained approximately 6×10^7 and 1×10^9 CFU/ml for yeast (OH 71.4, OH72.4, OH 181.1, and OH 182.9) and bacterial (AS43.3, AS 43.4, and OH 131.1) antagonists, respectively. The "Buffer" treatment was sprayed to run-off with weak buffer and Tween 80. All treatments were subjected to infection by ascospore inoculum from natural sources and *G. zeae* Z3639-colonized yellow dent corn.

[b] DS, disease severity; DI, disease incidence; 100-kw (g), 100 kernel weight; and DON (ppm), deoxynivalenol. Within a column means are compared using the $LSD_{(0.05)}$ value at the bottom of the column (Fisher's protected LSD, $P \leqslant 0.05$).

Table 4
Efficacy of antagonists grown in carbon:nitrogen 6.5 or 11.0 media in reducing FHB on cultivars Pioneer 2545 and Freedom at Peoria, IL, in a 2000 field experiment[a,b]

Treatment	Pioneer 2545				Freedom			
	DS (%)	DI (%)	100-kw (g)	DON (ppm)	DS (%)	DI (%)	100-kw (g)	DON (ppm)
No treatment	3.5	23.8	2.55	5.9	1.0	10.8	2.85	0.8
Buffer	7.6	32.1	2.57	5.3	0.6	7.9	2.71	2.2
AS 43.3 (6.5)	4.7	27.1	2.57	8.9	0.6	7.5	2.70	0.7
AS 43.3 (11.0)	4.3	26.7	2.49	5.2	0.9	10.0	2.79	1.1
AS 43.4 (6.5)	4.2	24.2	2.53	5.4	0.4	5.0	2.80	0.7
AS 43.4 (11.0)	4.4	24.6	2.46	6.9	1.1	10.8	2.68	1.2
OH 71.4 (6.5)	3.9	23.8	2.55	12.3	0.7	7.9	2.71	0.9
OH 71.4 (11.0)	3.3	21.2	2.61	4.8	0.8	10.0	2.70	1.6
OH 72.4 (6.5)	5.5	31.2	2.57	5.6	0.8	9.6	2.80	1.0
OH 72.4 (11.0)	5.0	27.5	2.55	7.1	0.9	10.8	2.80	0.7
OH 131.1 (6.5)	4.6	29.6	2.57	6.7	0.7	8.8	2.76	1.3
OH 131.1 (11.0)	4.9	26.2	2.58	3.8	0.8	10.4	2.71	1.2
OH 181.1 (6.5)	5.3	26.7	2.51	5.9	0.8	10.8	2.75	1.0
OH 181.1 (11.0)	3.2	18.8	2.68	4.7	0.9	9.6	2.86	0.7
OH 182.9 (6.5)	4.1	27.1	2.53	4.1	0.9	10.4	2.81	1.7
OH 182.9 (11.0)	3.0	20.9	2.56	5.2	0.9	11.7	2.82	6.2
$LSD_{(0.05)}$	1.8	7.7	0.08	5.1	0.5	5.3	0.07	3.2

[a] Wheat heads were sprayed to near run-off at the time of flowering with a suspension of 48-h-old antagonist-colonized broth diluted to 25% with weak PO_4 buffer and Tween 80 wetting agent. Microbial suspensions contained approximately 3×10^7 and 5×10^8 CFU/ml for yeast (OH 71.4, OH 72.4, OH 181.1, and OH 182.9) and bacterial (AS 43.3, AS 43.4, and OH 131.1) antagonists, respectively. The "Buffer" treatment was sprayed to runoff with weak buffer and Tween 80. All treatments were subjected to infection by ascospore inoculum from natural sources and *G. zeae*-colonized yellow dent corn.

[b] DS, disease severity; DI, disease incidence; 100-kw (g), 100 kernel weight; and DON (ppm), deoxynivalenol. Within a column means are compared using the $LSD_{(0.05)}$ value at the bottom of the column (Fisher's protected LSD, $P \leqslant 0.05$).

wetting agent control treatment also had one of the lowest disease severity ratings, a result in contrast to our other field trials where the buffer treated wheat control had equivalent or higher disease ratings than the untreated control. Loss of storage cooler refrigeration after obtaining 100-kernel weight caused stored

Table 5
Efficacy of antagonists grown in carbon:nitrogen 6.5 or 11.0 media in reducing FHB on cultivars Pioneer 2545 and Freedom at Wooster, OH, in a 2000 field experiment[a,b]

Treatment	Pioneer 2545				Freedom			
	DS (%)	DI (%)	100-kw (g)	DON (ppm)	DS (%)	DI (%)	100-kw (g)	DON (ppm)
No treatment	4.2	26.4	2.20	3.0	2.7	25.8	2.30	4.2
Buffer	8.4	40.0	2.10	3.9	3.0	24.7	2.26	3.9
AS 43.3 (6.5)	4.0	27.8	2.28	5.1	2.2	22.0	2.29	4.2
AS 43.3 (11.0)	4.1	30.3	2.18	4.3	4.0	30.3	2.22	4.6
AS 43.4 (6.5)	5.8	36.1	2.10	4.6	3.8	31.1	2.33	4.6
AS 43.4 (11.0)	4.2	28.3	2.18	5.0	5.0	34.2	2.12	5.6
OH 71.4 (6.5)	2.9	22.5	2.30	3.6	3.9	28.1	2.20	3.8
OH 71.4 (11.0)	4.1	24.2	2.18	3.9	2.9	25.8	2.26	3.8
OH 72.4 (6.5)	3.5	23.3	2.12	3.4	3.3	27.5	2.27	4.6
OH 72.4 (11.0)	2.9	23.1	2.18	3.5	3.7	30.6	2.18	3.6
OH 131.1 (6.5)	4.0	25.3	2.15	3.2	3.8	31.1	2.31	3.6
OH 131.1 (11.0)	3.9	24.2	2.26	5.3	2.2	21.7	2.24	3.6
OH 181.1 (6.5)	4.1	28.6	2.32	5.1	4.4	32.8	2.26	3.8
OH 181.1 (11.0)	5.6	32.5	2.25	5.2	3.4	27.5	2.26	4.8
OH 182.9 (6.5)	2.9	21.4	2.25	3.4	3.0	27.8	2.17	3.8
OH 182.9 (11.0)	3.4	22.0	2.25	2.5	2.7	25.3	2.25	3.4
$LSD_{(0.05)}$	1.3	6.4	0.06	1.5	1.1	6.5	0.06	1.4

[a] Wheat heads were sprayed to near run-off at the time of flowering with a suspension of 48-h-old antagonist-colonized broth diluted to 25% with weak PO_4 buffer and Tween 80 wetting agent. Microbial suspensions contained approximately 3×10^7 and 5×10^8 CFU/ml for yeast (OH 71.4, OH 72.4, OH 181.1, and OH 182.9) and bacterial (AS 43.3, AS 43.4, and OH 131.1) antagonists, respectively. The "Buffer" treatment was sprayed to runoff with weak buffer and Tween 80. All treatments were subjected to infection by ascospore inoculum from natural sources and *G. zeae*-colonized yellow dent corn.

[b] DS, disease severity; DI, disease incidence; 100-kw (g), 100 kernel weight; and DON (ppm), deoxynivalenol. Within a column means are compared using the $LSD_{(0.05)}$ value at the bottom of the column (Fisher's protected LSD, $P \leqslant 0.05$).

grain to become moist and negated obtaining accurate DON content values.

3.3. Effect of antagonist dosage:1999 Peoria, IL, and Wooster, OH, field tests

Factorial analysis of data after removing control values often resulted in significant interactions between the factors "antagonist" and "dose." Therefore, the overall influence of dose or antagonist was only obtained occasionally. For results in Peoria on cultivar Pioneer 2545, antagonist and dose significantly interacted for both severity and incidence data ($P = 0.01$ and $P = 0.0001$, respectively). Wheat treated with five of the seven antagonists tested had reduced FHB disease incidence for wheat cultivar Pioneer 2545 at one or both of the concentrations assayed over the "no treatment" control ($P \leqslant 0.05$, Fig. 1) but only AS 43.4 treated wheat had reduced disease severity compared to the "no treatment" control, and only at the 10% dose (data not shown). Overall disease severity was low (<2%). Antagonist treated wheat rarely had differing 100 kernel weights (data not shown).

On cultivar Freedom in Peoria, antagonist by dose interactions were not significant for severity ($P = 0.42$) or incidence ($P = 0.49$) data. However, antagonists did not differ in their overall affect on severity ($P = 0.08$) or incidence ($P = 0.26$). Similarly, the two dosages tested did not differ in their overall affect on severity ($P = 0.35$) or incidence ($P = 0.93$). Wheat treated with antagonists, except for OH 131.1, had reduced FHB incidence compared to the "no treatment" and buffer controls, at one or both of the antagonist concentrations assayed (Fig. 2). Wheat treated with antagonists showed no reduction in disease severity (data not shown). Overall disease severity was low (<1.6%). Wheat treated with yeasts OH 71.4 and OH 72.4 and bacteria OH 131.1 had increased 100 kernel weights at one of the dosages tested (data not shown). For results in Wooster on cultivar Pioneer 2545, antagonist and dose treatments significantly interacted for both severity and incidence data ($P = 0.0001$ and $P = 0.0001$, respectively). Treatment with 10% doses of bacterial antagonist OH 71.4 and yeast antagonist OH 182.9 resulted in wheat with disease severity reduced by 53 and 55%, respectively, compared to the "no treatment" control. Wheat treated with antagonists OH 131.1, OH 181.1 and OH 182.9 had reduced disease severity for both of the concentrations tested (Table 3). Wheat treated with antagonists, except bacterial strain treatments AS 43.3 and AS 43.4, had reduced disease incidence at one or both of the dosages tested (Table 3). Compared to the buffer control, all antagonist treated wheat had increased 100 kernel weight while one concentration of OH 72.4 and OH 182.9 treated wheat had reduced DON content in grain (Table 3).

Antagonist and dose also significantly interacted for both severity and incidence data ($P = 0.001$ and $P = 0.0001$, respectively) in results on cultivar Freedom at Wooster, Ohio. Disease severity and incidence was less on cultivar Freedom than Pioneer 2545 (Table 3). Treatment with one dosage of three of the seven antagonists resulted in wheat with reduced disease severity compared to the buffer control (Table 3). No antagonists reduced incidence or DON and none increased the 100 kernel weight of treated wheat. One dose of two of the antagonist treatments displayed an increase in FHB incidence (Table 3).

3.4. C:N ratio of the production media for antagonists: 2000 field trials at Peoria, IL, and Wooster, OH

Factorial analysis of data after removing control values occasionally resulted in significant interactions between the factors "antagonist" and "C:N." On cultivar Pioneer 2545 in Peoria, antagonist by C:N interactions were not significant for severity ($P = 0.43$) or incidence ($P = 0.76$) data. Antagonist treatments did not differ in their overall effect on severity ($P = 0.11$) or incidence ($P = 0.09$). Wheat treated with antagonists produced in C:N 11.0 medium had less FHB incidence ($P = 0.02$; C:N 11.0 = 23.7, C:N 6.5 = 27.1) but not severity ($P = 0.06$). Wheat treated with all antagonist by C:N combinations had reduced disease severity compared to the buffer control. Treatment with yeast OH 182.9 produced in C:N 11.0 medium resulted in wheat with 61% less FHB severity (Table 4). Wheat treated with four of the antagonist treatments had reduced disease incidence when the antagonists were produced in one or both media. Wheat treated with antagonists did not have reduced severity or incidence when compared to the "no treatment" control. Antagonist treated wheat rarely had differing 100 kernel weight or DON content (Table 4).

On cultivar Freedom in Peoria, antagonist by C:N interactions were not significant for severity ($P = 0.42$) or incidence ($P = 0.72$) data. Antagonist treatments did not differ in their overall effect on severity ($P = 0.93$) or incidence ($P = 0.71$). Wheat treated with antagonists produced in C:N 6.5 medium had a greater reduction in FHB severity ($P = 0.03$; C:N 11.0 = 0.9, C:N 6.5 = 0.7) but not in incidence ($P = 0.06$) compared to wheat treated with antagonists produced in C:N 11.0 medium. Overall severity and incidence were less on cultivar Freedom than on cultivar Pioneer 2545 (Table 4). None of the antagonist by C:N combination treatments reduced disease severity or incidence on cultivar Freedom but wheat treated with five of the seven antagonist treatments had increased 100 kernel weight compared to the buffer control. DON was higher in OH 182.9 treated wheat when the antagonist was produced in C:N 11.0 medium (Table 4).

On cultivar Pioneer 2545 in Wooster, antagonist by C:N interactions were significant for severity ($P = 0.01$) but not for incidence ($P = 0.24$) data. The C:N of the production media did not influence the effect of antagonists on disease incidence ($P = 0.95$). Wheat treated with antagonists differed in disease incidence (32.2, 30.6, 29.0, 24.7, 23.3, 23.2, and 21.7 for AS 43.4, OH 181.1, AS 43.3, OH 131.1, OH 71.4, OH 72.4, and OH 182.9, respectively; $P = 0.0001$, $LSD_{(0.05)} = 4.6$). Wheat treated by all antagonists by C:N combinations had reduced disease severity compared to the buffer control but rarely compared to the "no treatment" control (Table 5). Wheat treated with antagonists displayed increased 100 kernel weight when the antagonist was produced on at least one of the C:N media while DON was not influenced (Table 5).

On cultivar Freedom in Wooster, antagonist by C:N interactions were significant for severity ($P = 0.0002$) and incidence ($P = 0.005$) data. Antagonist by C:N combinations did not decrease and in rare cases increased disease severity and incidence (Table 5). Wheat treated with antagonists had increased or decreased 100 kernel weight depending on the specific antagonist by C:N treatment. Treatment of wheat with antagonist AS 43.4 produced in C:N 11.0 medium resulted in increased DON content but all other antagonist by C:N combinations had no affect on DON (Table 5).

4. Discussion

Biological control of Fusarium head blight was demonstrated in the field on a variety of hard red spring and soft red winter wheat cultivars at field locations in Langdon, ND, Peoria, IL, and Wooster, OH. Yeast strains *Cryptococcus* sp. OH 71.4, *C. nodaensis* OH 182.9, and *Cryptococcus* sp. OH 181.1 reduced disease severity by 50–60% in several field trials (Tables 2–4 on cultivar Pioneer 2545). However, the effectiveness of biological control varied at times depending on the antagonist dose, the carbon to nitrogen ratio of the antagonist production medium and the wheat cultivar utilized in the field tests.

A higher dose of antagonist was beneficial, deleterious or of no effect in reducing FHB disease depending on the specific antagonist and disease parameter considered. Yeast OH 71.4, for instance, was more effective in reducing FHB severity at the lower CFU concentration tested while the converse was true for yeast OH 72.4 (Table 3 on cultivar Pioneer 2545). Overall dose effects could not be determined due to interactions with antagonists (1999 Peoria tests on cultivar Pioneer 2545 and 1999 Wooster tests on cultivars Pioneer 2545 and Freedom) or were not significantly different (1999 Peoria test on cultivar Freedom). Below a threshold saturation dose, incremental differences in biological control effect are often seen with increasing antagonist dose (Raaijmaker et al., 1995; Schisler et al., 1997). A clear differ-

ence in the efficacy of 10 and 50% fully colonized broth for our antagonists was not seen in this study. Variability of results depending on experimental site and wheat cultivar utilized contributed to difficulties in determining a consistent dose–response for the antagonists. Further field testing with decreased dosages that are divided into several dose increments may help determine what antagonist dose is optimal for maximizing disease reduction without being excessive.

Though antagonists frequently were effective in reducing the effect of Fusarium head blight in the field, the carbon to nitrogen ratio (C:N) of the antagonist production medium frequently interacted statistically with the antagonist factor. In the rare cases where the interaction was not significant, antagonist cells produced in C:N 6.5 medium were more effective in one case (Peoria, cultivar Freedom, disease severity data) but less effective in another (Peoria, cultivar Pioneer 2545, incidence data). In greenhouse studies conducted on durum wheat (Schisler et al., 2002), there also was no consistent trend between biological control agent performance and the liquid culture medium used to produce it. Microbial culture production environments can influence the performance of biological control agents (Ibrahim et al., 2002; Schisler et al., 1998, 1991, 2000a,b; Slininger et al., 1994). Evaluating the influence of additional liquid culture production environments, such as increased C:N ratios and carbon loading, on biological control agent performance against FHB may still result in implicating this factor's importance.

On hard red spring wheat, five of six antagonists were effective in reducing disease severity on cultivar Grandin but only two antagonists were on cultivar Russ. Similar trends were seen with antagonists frequently reducing disease symptoms on the soft red winter wheat Pioneer 2545 but rarely on Freedom. Biocontrol agents have previously been shown to differ in the level of control exhibited on different host cultivars (King and Parke, 1993) and in their performance relative to other agents when tested on more than one cultivar (Schisler et al., 2000b). The lowest levels of disease severity and incidence tended to occur when biological control was combined with the use of the resistant cultivar Freedom (Tables 3 and 4). While this result suggests the potential benefit of multiplexing these FHB control measures, experiments designed with wheat cultivars as a treatment would be necessary to confirm this trend in the data. The reduction of DON content in FHB grain continues to be elusive. DON content was rarely reduced by biological control treatments in this study, even when disease severity and incidence parameters were significantly reduced. Because recent field studies in additional sites have shown reduced levels of DON in biological control treatments where severity and incidence were reduced (Schisler, unpublished), the utility of biological control to reduce DON in FHB fields remains uncertain.

The purpose of this study was not to determine which biological control strain was superior but rather to maximize each strain's performance via management of antagonist dose and production medium. Attempts to rank each strain's best performance for each of the field studies resulted in no statistical separation of antagonist ranks based on severity ($P = 0.11$) or incidence ($P = 0.43$) (Kruskal–Wallis One-Way Nonparametric ANOVA). However, the possibility of commercially developing a biological control product active against FHB requires restricting the number of strains that proceed to the stage of large-scale production and field testing. Therefore, while rank data were not separable statistically, the selection of *C. nodaensis* OH 182.9 and *Cryptococcus* sp. OH 71.4 for further development can be justified based on these strains' top two rankings for reducing both FHB severity and incidence. Initial studies also support the amenability of OH 182.9 to mass production while maintaining efficacy against FHB in field environments (Schisler et al., 2001).

Despite the fact that field environments are variable and challenging for successfully employing biological control measures against plant diseases, encouraging levels of reduction of Fusarium head blight were observed. Further improvements in the level of control achieved using these antagonists will be sought via additional research on optimizing media and formulations. Additional efficacy gains will also be attempted via the selection of fungicide insensitive variants of selected antagonists and their use in combination with fungicides registered for use against FHB. Because combinations of antagonists are frequently effective in enhancing biological control efficacy and consistency (Guetsky et al., 2001; Raupach and Kloepper, 1998; Schisler et al., 1997), antagonist combinations also will be tested for utility in enhancing FHB biocontrol.

Disclaimer

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References

Bai, G., Shaner, G., 1994. Scab of wheat: prospects for control. Plant Dis. 78, 760–766.

Bai, G.-H., Shaner, G., Ohm, H., 2000. Inheritance of resistance to *Fusarium graminearum* in wheat. Theor. Appl. Genet. 100, 1–8.

Beker, M.J., Rapoport, A.I., 1987. Conservation of yeasts by dehydration. Adv. Biochem. Engineer. Biotechnol. 35, 128–171.

Boehm, M.J., Khan, N.I., Schisler, D.A., 1999. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight: field testing of antagonists. In: Wagester, J.A., Ward, R., Hart, L.P., Hazen, S.P., Lewis, J., Borden, H. (Eds.), 1999 National Fusarium Head Blight Forum Compilers. University Printing, Michigan State University, East Lansing, MI, pp. 56–59.

Bujold, I., Paulitz, T.C., Carisse, O., 2001. Effect of Microsphaeropsis sp. on the production of perithecia and ascospores of *Gibberella zeae*. Plant Dis. 85, 977–984.

Bushnell, W.R., Somers, D.A., Giroux, R.W., Szabo, L.J., Zeyen, R.J., 1998. Genetic engineering of disease resistance in cereals. Can. J. Plant Pathol. 20, 137–149.

Casale, W.L., Hart, L.P., 1988. Inhibition of ^{3}H-leucine incorporation by trichothecene mycotoxins in maize and wheat tissue. Phytopathology 78, 1673–1677.

da Luz, W.C., 1988. Biocontrol of fungal pathogens of wheat with bacteria and yeasts. In: Proceedings of the 5th International Congress of Plant Pathology, Kyoto, Japan, p. 348.

Dill-Macky, R., Jones, R.K., 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84, 71–76.

Fernandez, M.R., 1992. The effect of *Trichoderma harzianum* on fungal pathogens infesting wheat and black oat straw. Soil Biol. Biochem. 24, 1031–1034.

Forsyth, O.M., Yoshizawa, T., Morooka, N., Tuite, J., 1977. Emetic and refusal activity of deoxynivalenol to swine. Appl. Environ. Microbiol. 34, 547–552.

Fuming, D., Shimin, Z., Beiqi, Z., An, Z., 1994. Control effects of four bacterial strains on wheat scab (*Fusarium graminearum*). Acta Agr. Shang. 10, 59–63.

Guetsky, R., Shtienberg, D., Elad, Y., Dinoor, A., 2001. Combining biocontrol agents to reduce the variability of biocontrol. Phytopathology 91, 621–627.

Hawk, A.L., 1988. Mycotoxins in grain marketing. In: Proceedings of the 53rd Annual Corn and Sorghum Research Conference, Chicago, IL, pp. 299–303.

Homdork, S., Fehrmann, H., Beck, R., 2000. Influence of different storage conditions on the mycotoxin production and quality of *Fusarium*-infected wheat grain. J. Phytopathol. 148, 7–15.

Ibrahim, L., Butt, T.M., Jenkinson, P., 2002. Effect of artificial culture media on germination, growth, virulence and surface properties of the entomopathogenic hyphomycete *Metarhizium anisopliae*. Mycol. Res. 106, 705–715.

Johnson, K.B., 1994. Dose–response relationships and inundative biological control. Phytopathology 84, 780–784.

Johnston, H.W., 1994. Resistance in advanced winter wheat breeding lines to scab, 1993. Biol. Cultural Tests 9, 119.

Jones, R.K., 1999. Seedling blight development and control in spring wheat damaged by *Fusarium graminearum* Group 2. Plant Dis. 83, 1013–1018.

Jones, R.K., 2000. Assessments of Fusarium head blight of wheat and barley in response to fungicide treatments. Plant Dis. 84, 1021–1030.

Khan, N.I, Schisler, D.A, Boehm, M.J., Slininger, P.J., Bothast, R.J., 2001. Selection and evaluation of microorganisms for biocontrol of Fusarium head blight of wheat incited by *Gibberella zeae*. Plant Dis. 85, 1253–1258.

King, E.B., Parke, J.L., 1993. Biocontrol of Aphanomyces root rot and Pythium damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Dis. 77, 1185–1188.

Large, E.C., 1954. Growth stages in cereals. Illustrations of the Feekes scale. Plant Pathol. 3, 128–129.

McMullen, M., Jones, R., Gallenberg, D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81, 1340–1348.

Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Seaman, W.L., Seifert, K.A., Turkington, K., Voldeng, H., 1998. Effect of tillage practice on Fusarium head blight of wheat. Can. J. Plant Pathol. 20, 95–103.

Perondi, N.L., da Luz, W.C., Thomas, R., 1996. Controle microbiológico da giberela do trigo. Fitopatol. Brasiliera 21, 243–249.

Pirgozliev, S.R., Edwards, S.G., Hare, M.C., Jenkinson, P., 2002. Effect of dose rate of azoxystrobin and metconazole on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain. Eur. J. Plant Pathol. 108, 469–478.

Proctor, R.H., Hohn, T.M., McCormick, S.P., 1995. Reduced virulence of *Gibberella zeae* caused by disruption of trichothecene toxin biosynthesis gene. MPMI 8, 593–601.

Raaijmaker, J.M., Leeman, M., van Oorschot, M.M.P., van der Sluis, I., Schippers, B., Bakker, P.A.H.M., 1995. Dose–response relationships in biological control of Fusarium wilt of radish by *Pseudomonas* spp. Phytopathology 85, 1075–1081.

Raupach, G.S., Kloepper, J.W., 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88, 1158–1164.

Sato, I., Kobayasi, H., Hanya, Y., Abe, K., Murakami, S., Scorzetti, G., Fell, J.W., 1999. *Cryptococcus nodaensis* sp. nov., a yeast isolated from soil in Japan that produces salt-tolerant and thermostable glutaminase. J. Industr. Microbiol. Biotechnol. 22, 127–132.

Schisler, D.A., Burkhead, K.D., Slininger, P.J., Bothast, R.J., 1998. Selection, characterization and use of microbial antagonists for the control of Fusarium dry rot of potatoes. In: Boland, G.J., Kuykendall, L.D. (Eds.), Plant–Microbe Interactions and Biological Control. Marcel Dekker, New York, pp. 199–222.

Schisler, D.A., Jackson, M.A., Bothast, R.J., 1991. Influence of nutrition during conidiation of *Colletotrichum truncatum* on conidial germination and efficacy in inciting disease in *Sesbania exaltata*. Phytopathology 81, 587–590.

Schisler, D.A., Khan, N.I., Boehm, M.J., Lipps, P.E., 2000a. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight: field tests of antagonists in 2000. In: Ward, R.W., Canty, S.M., Lewis, J., Siler, L. (Eds.), 2000 National Fusarium Head Blight Forum, Kinko's, Okemos, MI, pp. 105–109.

Schisler, D.A., Khan, N.I., Boehm, M.J., Slininger, P.J., 2002. Greenhouse and field evaluation of biological control of Fusarium head blight on durum wheat. Plant Dis. 86, 1350–1356.

Schisler, D.A., Khan, N.I., Iten, L.B., Boehm, M.J., 2001. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight: pilot-plant-scale production and processing of biomass of yeast antagonists. In: Canty, S.M., Lewis, J., Siler, L., Ward, R.W. (Eds.), 2001 National Fusarium Head Blight Forum, Kinko's, Okemos, MI, pp. 87–90.

Schisler, D.A., Slininger, P.J., Bothast, R.J., 1997. Effects of antagonist cell concentration and two-strain mixtures of biological control of Fusarium dry rot of potatoes. Phytopathology 87, 177–183.

Schisler, D.A., Slininger, P.J., Hanson, L.E., Loria, R., 2000b. Potato cultivar, pathogen isolate, and antagonist cultivation medium influence the efficacy and ranking of bacterial antagonists of Fusarium dry rot. Biocontrol Sci. Technol. 10, 267–279.

Slininger, P.J., Schisler, D.A., Bothast, R.J., 1994. Two-dimensional liquid culture focusing: a method of selecting commercially promising microbial isolates with demonstrated biological control capability. In: Ryder, M.H., Stephens, P.M., Bowen, G.D. (Eds.), Improving Plant Productivity with Rhizosphere Bacteria. 3rd International Workshop on Plant Growth-Promoting Rhizobacteria, Adelaide, S. Australia. Graphic Services, Adelaide, Australia. CSIRO Division of Soils, Glen Osmond, pp. 29–32.

Smith, P.K, Handelsman, J., Goodman, M.R., 1997. Modeling dose–response relationships in biological control: partitioning host responses to the pathogen and biocontrol agent. Phytopathology 87, 720–729.

Snijders, C.H.A., 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. Neth. J. Plant Pathol. 96, 187–198.

Stack, R.W., McMullen, M.P., 1995. A visual scale to estimate severity of Fusarium head blight in wheat. N.D. State Univ. Ext. Serv. Bull., p. 1095.

Suty, A., Mauler-Machnik, A., 1997. Fusarium ear blight on wheat-epidemiology and control of *Gibberella zeae*, the teleomorph of *Fusarium graminearum* with Folicur. In: Dehne, H.-W., Adam, G., Diekmann, M., Frahm, J., Mauler-Machnik, A., Van Haltreren, P. (Eds.), Diagnosis and Identification of Plant Pathogens, Proceedings of the 4th International Symposium of the European Foundation for Plant Pathology. Kluwer Academic Publishers, Dordrecht, pp. 243–246.

Teich, A.H., Hamilton, J.R., 1985. Effect of cultural practices, soil phosphorus, potassium, and pH on the incidence of Fusarium head blight and deoxynivalenol levels in wheat. Appl. Environ. Microbiol. 49, 1429–1431.

Vesonder, R.F., Hesseltine, C.W., 1980. Vomitoxin: natural occurrence on cereal grains and significance as a refusal and emetic factor to swine. Process Biochem. 16, 12–15.

Vesonder, R.F., Ciegler, A., Jensen, A.H., Rohwedder, W.K., Weislander, D., 1976. Co-identity of the refusal and emetic principle from Fusarium-infected corn. Appl. Environ. Microbiol. 31, 280–285.

Wilcoxson, R.D., 1996. Fungicides for control of Fusarium head blight. Int. J. Tropical Plant Dis. 14, 27–50.

Windels, C.E., 2000. Economic and social impacts of Fusarium head blight: Changing farms and rural communities in the Northern Great Plains. Phytopathology 90, 17–21.